J. Physiol. (1978), **282**, *pp.* 345–352
With 6 text-figures
Printed in Great Britain

THE INTERACTIONS OF PROTONS, CALCIUM AND POTASSIUM IONS ON CARDIAC PURKINJE FIBRES

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(*Received 24 February 1977*)

SUMMARY

1. In sheep Purkinje fibres, acidosis shifts the steady-state current–voltage relation in an inward direction over a wide range of negative potentials. The reversal potential for i_{K_2} is shifted in a positive direction. These changes are consistent with K^+ accumulation in the extracellular spaces of the Purkinje fibre.

2. These effects of acidosis are completely prevented by increasing $[K]_o$ from the normal range (2·7–5·4 mM) to 10·8 mM.

3. Increasing $[Ca]_o$ from 2 to 6 mM also produces an inward shift in the steady-state current–voltage relation and a positive shift of the i_{K_2} reversal potential.

4. In 10·8 mM-$[K]_o$ these effects are absent even when $[Ca]_o$ is increased to 20 mM.

5. One possible explanation is that K^+ ions may protect the Na pump from inhibition by protons and Ca^{2+} ions. Alternative explanations are also discussed.

INTRODUCTION

The effects of pH and Ca^{2+} ions on the Na threshold and on the activation range of the pace-maker K current, i_{K_2}, were reported in the previous paper (Brown & Noble, 1978). The results suggest that the membrane surface negative potential of the Purkinje fibre is nearly -20 mV. In that paper it was also noted that pH influences the steady-state current–voltage relations. These effects, and those of Ca, are however more complex and more difficult to interpret than those on activation threshold. This complexity is not unexpected since the net current at any potential is a balance between a number of inward and outward currents so that the net effects of Ca^{2+} and H^+ must also depend on their actions on each individual membrane current.

In this paper we have studied the effects of pH and Ca^{2+} on the net steady state membrane current over a wide range of potentials. The results suggest that changes in the K^+ balance in the clefts of the Purkinje fibre may be involved in mediating some of the effects. We have also found that the effects are strongly dependent on the level of K^+ in the bathing solution. This dependence shows a striking parallel to the known clinical results on the interactions of the three ions involved.

METHODS

The methods were the same as Cohen, Daut & Noble (1976). The pH of the Tyrode solutions was altered by changing the bathing bicarbonate concentration. The pH on the solutions was then checked with a Beckman pH meter.

It is well known that ionized [Ca^{2+}] varies as a function of the pH of the solution when a bicarbonate buffer is used. In order to control for this systematic increase in free [Ca^{2+}] at reduced pH, the concentration of Ca^{2+} added to the Tyrode was reduced by up to 20% in solutions of lowered pH in some experiments. The experimental results were indistinguishable in the presence or absence of this small control change in [Ca^{2+}].

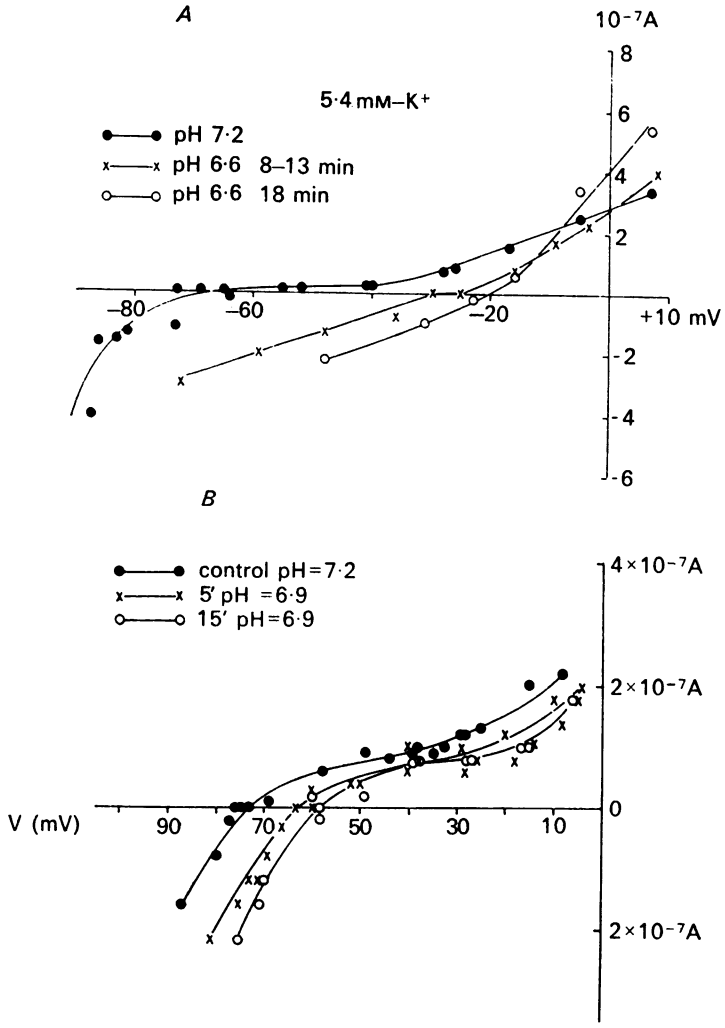

Fig. 1. Influence of acidosis on steady-state current–voltage relations. *A*, change from pH 7·2 to 6·6 at $[K]_o$ = 5·4 mM. *B*, change from pH 7·2 to 6·9 at $[K]_o$ = 5·4 mM.

RESULTS

Influence of acidosis on steady-state current–voltage curves

When Purkinje fibres were exposed to acid solutions containing K concentrations within the normal range (2·7–5·4 mM) the current–voltage diagram was found to shift progressively in an inward direction. Fig. 1*A* shows the result of exposure to pH 6·6 at a K^+ concentration of 5·4 mM. There is a large membrane depolarization and, at potentials negative to −10 mV, the current changes in an inward direction. After 18 min the acid curve is found to cross the control curve at about −10 mV. Fig. 1*B* shows a similar result obtained on exposure to pH 6·9.

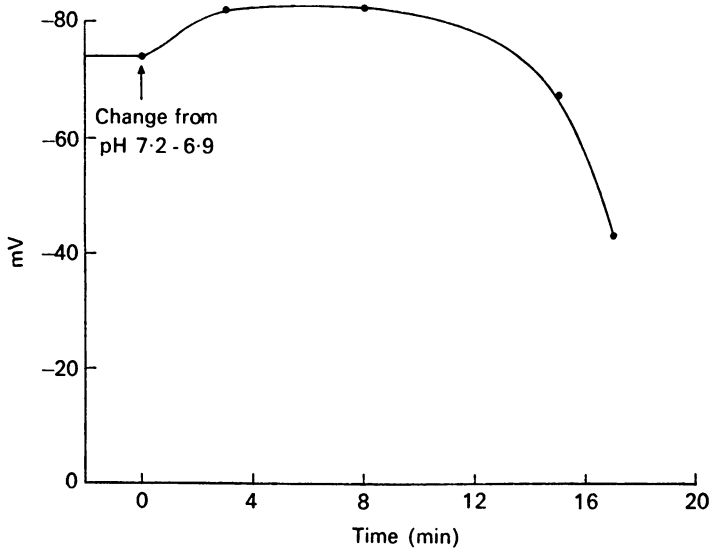

Fig. 2. Influence of acidosis (change from pH 7·2 to 6·9) on resting potential.

The development of depolarization following mild acidosis sometimes occurs very slowly. Fig. 2 shows the resting potential of a Purkinje fibre following exposure to pH 6·9. Marked depolarization appears in this case only after 15 min. During the first 8 min there is a small hyperpolarization.

By contrast, the effects of strong acidosis (pH 4·0 or 5·0) were found to be virtually immediate (Brown, 1973).

Exposure to alkaline solutions had virtually no effect on the steady-state current–voltage relations (Brown, 1973).

Change in i_{K_2} reversal potential during acidosis

In the preceding paper (Brown & Noble, 1978, Fig. 5) a small, though not necessarily significant, change in E_{rev} for i_{K_2} was detected in acid solution. During prolonged exposure, the change becomes very marked. Fig. 3 shows an experiment performed in 3·6 mM-K^+. At pH 7·2, the reversal potential for i_{K_2} lies at −105 mV. Following exposure to pH 7·0 there is a large inward shift in the current–voltage diagram as already shown in Fig. 1. Due to the progressive deterioration of the fibre

and the large currents required to hyperpolarize the membrane, it was not possible to determine an exact value for E_{rev} at pH 7·0, but its value was shown to lie between −84 and −72 mV, a positive shift of at least 20 mV.

These results are similar to those obtained during pump inhibition by cardiac glycosides (Cohen *et al.* 1976), where the major part of the change in E_{rev} was found to be attributable to an increased K^+ concentration, $[K^+]_c$, in the extracellular

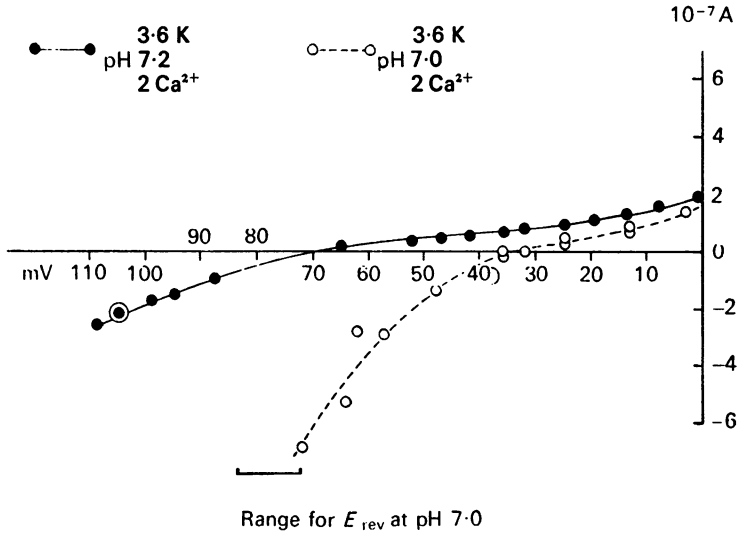

Fig. 3. Influence of mild acidosis (change from pH 7·2 to 7·0) at $[K]_o$ = 3·6 mM. Before acidosis E_K lies at −105 mV (●). After acidosis E_K shifts to between −84 and −72 mV (——).

clefts of the preparation. A concomitant change in this case is a progressive increase in the ratio of time-independent to time-dependent current change during hyperpolarizing clamp pulses (see Cohen *et al.* 1976, Fig. 10). Similar results are obtained during acidosis.

Comparable results were also obtained in experiments involving voltage clamp pulses in the plateau range of potentials. Following acidosis, the instantaneous current jump is increased while the time-dependent change (i_x in this range) is reduced. It was also found that the inward Ca current and the fibre contractility were reduced (Brown, 1973; see also Wada & Goto, 1975).

Protection against acidosis by high K solution

The effects of acidosis at normal K^+ concentrations strongly resemble those produced during pump inhibition by toxic doses of cardiac glycosides. Since these effects may be countered by increasing the extracellular K^+ concentration, we decided to test whether K^+ ions may also protect against acidosis.

Fig. 4 shows the result of exposing a Purkinje fibre to pH 6·6 when the bathing K^+ concentration is increased to 10·8 mM. The effects of acidosis are completely suppressed. There is no significant change in the steady-state current–voltage diagram and the potassium current reversal potential remains virtually constant at −95 mV.

The effects of increased extracellular Ca^{2+}

The effect of raising the extracellular Ca^{2+} concentration from 2 to 6 mM is shown in Fig. 5. There is an inward shift of current at all potentials which is completely reversible on returning to 2 mM-Ca.

We attempted to measure the effect of increased $[Ca^{2+}]_o$ on E_{rev} for i_{K_2} but these experiments were often complicated by the appearance of transient inward (TI) currents superimposed on the K decay tails (Ferrier & Moe, 1973; Lederer &

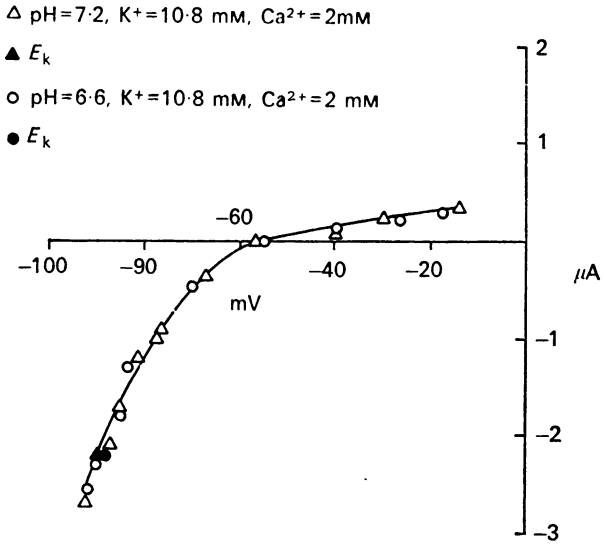

Fig. 4. Protection against effects of acidosis produced by high $[K]_o$. At 10·8 mM, a change to pH 6·6 no longer shifts E_K.

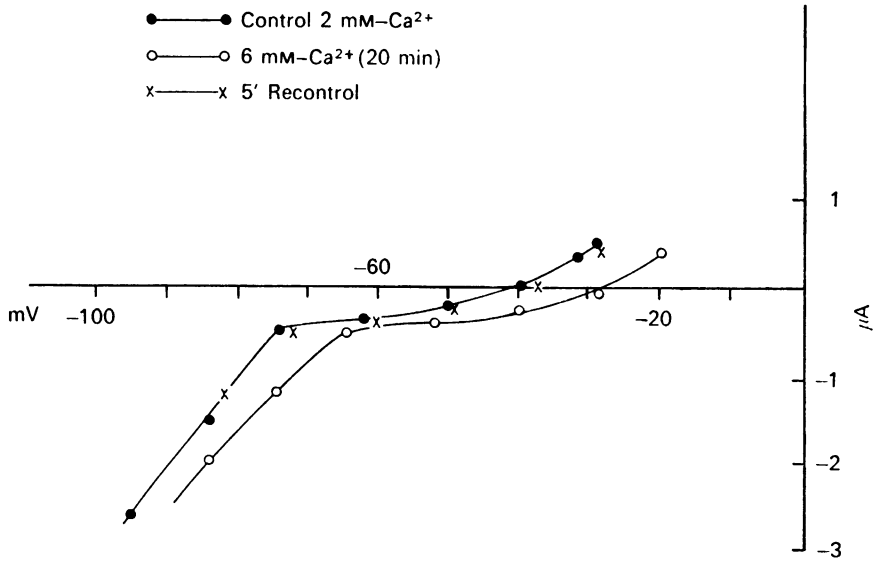

Fig. 5. Influence of increased extracellular Ca^{2+} concentration on steady-state current–voltage relation.

Tsien, 1976) so that the early part of the potassium current decay was obscured. In one experiment in which E_{rev} was followed during a rise in extracellular [Ca] from 2 to 12 mM a 26 mV positive shift in E_{rev} accompanied the inward shift in the current–voltage relationship. Di Francesco & McNaughton (1977) have also observed a positive shift in i_{K_2} reversal potential in high Ca solution.

The effects of increased $[Ca]_o$ *at high* $[K]_o$

As with the effects of acidosis, the effects of hypercalcaemia resemble some of the effects of pump inhibition (see Isenberg & Trautwein, 1974, Fig. 3; Cohen *et al.* 1976, Fig. 6). It therefore seemed important to perform the same experiments in

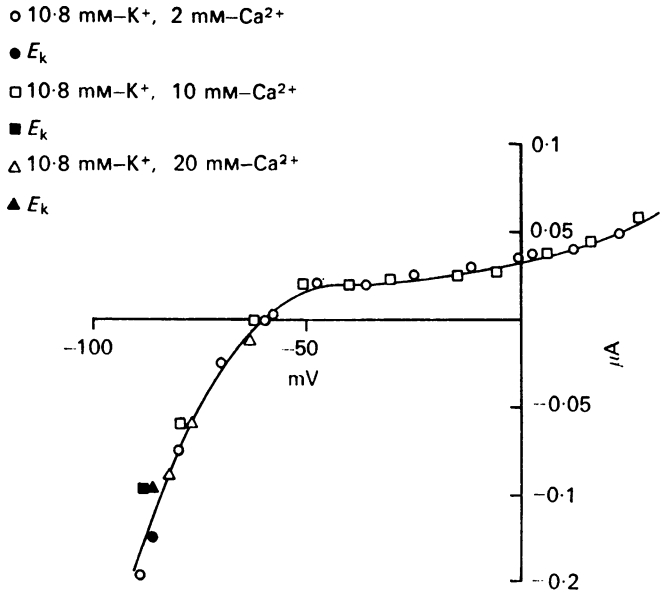

Fig. 6. Protection against effects of high Ca concentration produced by increasing $[K]_o$ to 10·8 mM.

K-rich solutions. The results of such an experiment are shown in Fig. 6. At a bathing $[K]^+$ of 10·8 mM, raising $[Ca^{2+}]_o$ from 2 to 10 mM and even to 20 mM has little or no effect on E_{rev} or on the steady-state current–voltage relation.

In three experiments at elevated $[Ca]_o$ and elevated $[K]_o$ there was either no change in the steady-state current–voltage relationship or else a slight *decrease* in inward current at potentials hyperpolarized to the initial resting potential. No change in the reversal potential of i_{K_2} was observed.

DISCUSSION

The results we have described in this paper suggest that changes in extracellular K^+ concentration in the restricted extracellular spaces of the Purkinje fibre might be responsible for mediating some of the changes in the steady-state current–voltage relationship and reversal potential for i_{K_2} upon application of solutions containing elevated concentrations of Ca^{2+} or H^+. The idea that extracellular K^+

concentrations in the Purkinje fibre or indeed other areas of the heart can be affected by changes in ions or drugs is not new (Maughan, 1973; Noble, 1976; Cohen *et al.* 1976; Baumgarten & Isenberg, 1977), and recent investigations with K^+ selective micro-electrodes have shown substantial increases in cleft potassium in rabbit atrium when the pH is lowered from 7·5 to 6·8 and then 6·1 (Skinner & Kunze, 1976).

It therefore seems useful to consider the possible alternatives by which K^+ accumulation can be made to occur. The K^+ in the clefts of the Purkinje fibre is determined by the balance of the transmembrane K^+ diffusion, K^+ ion pumping, and diffusion of K^+ between the cleft and bulk solutions. We will consider each possibility in turn.

The pump hypothesis

This argument suggests that since the results of the experiments reported here closely resemble those produced by inhibitory doses of ouabain (Cohen *et al.* 1976; Isenberg & Trautwein, 1974), one possible explanation of the results is that, like ouabain, Ca^{2+} and H^+ can inhibit the Na^+-K^+ exchange pump. This inhibition might occur by direct action on the Na^+-K^+ ATPase as reported by Balasubramanian, McNamara, Singh & Dhalla (1973) for rat heart muscle, or alternatively inhibition can occur by alteration of the local K^+ through a decrease in the membrane surface potential. In both cases the net result would be to decrease the inward movement of K^+ from the clefts to the intracellular space, and in the absence of alternative compensations for this change in pumping, accumulation will occur.

One argument against this hypothesis is that, in the red cell, the most carefully studied Na^+-K^+ ATPase does not show a substantial variation of activity in the experimental range of pH. This may represent differences in the Na^+-K^+ ATPase in heart and the red blood cell (see Balasubramanian *et al.* 1973). Alternatively it may represent differences in the membrane environment of the two ATPases. Too little is known about the Na^+–K^+ exchange pump in cardiac muscle to decide between these possibilities.

The membrane conductance hypothesis

This argument requires that Ca^{2+} and/or H^+ alters the passive membrane K^+ flux by changing the magnitude or voltage dependence of existing K^+ conductances or alternatively initiating new conductances. There has been substantial investigation of the role of Ca^{2+} in the control of K^+ permeability in the heart (Isenberg, 1975; Kass & Tsien, 1975; Bassingthwaite, Fry & McGuigan, 1976). However, no conclusive experiments to verify or reject this hypothesis have been performed. The equivocal nature of the results is at least partly due to the inability to distinguish between effects on conductance, and those on pumping or cleft architecture, as all lead to changes in cleft K^+, which then further alters both the membrane K^+ conductance and the Na^+-K^+ pump activity.

Cleft architecture hypothesis

It is always possible that the restriction to diffusion in the clefts is caused by a series of large molecules with substantial negative charge. In this case the restriction to diffusion can be reduced by reducing the negative charge on these molecules. If the cleft concentration of K^+ was initially below that in the bulk solution then

accumulation of K^+ will result in a $[K^+]$ nearer that in the bulk, and above that in the control solution.

Protection by elevated K^+ against the alterations produced by Ca^{2+} and H^+

Experimentally it is clear that increasing the Ca^{2+} or lowering the pH has a qualitatively different effect at low or high $[K^+]_b$. Unfortunately this does not help us to distinguish among the previously listed alternatives. For example elevated K^+ may prevent pump blockage caused by surface potential changes, or may increase the control K^+ conductance such that no further increase in conductance is possible. Still another alternative is that K^+ may alter the binding characteristics of cleft molecules, and so reduce restriction to diffusion.

However, the existence of this experimental result is interesting, even in the absence of a complete explanation, because of the striking parallel between the interactions of Ca^{2+}, H^+ and K^+ seen in voltage clamp, and the clinically observed interactions between these ions.

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